Facultat de Veterinària
Universitat Autònoma de Barcelona

The BLINK REFLEX: Comparative Electrophysiologic Study in the Domestic Species

Sònia Añor i Torres

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Universitat Autònoma de Barcelona
Facultat de Veterinària
Departament de Medicina i Cirurgia Animals

THE BLINK REFLEX: COMPARATIVE ELECTROPHYSIOLOGIC STUDY IN THE DOMESTIC SPECIES

Tesi que presenta Sònia Añor i Torres

per optar al títol de Doctora.

Directors de la tesi:

Martí Pumarola i Batlle

Universitat Autònoma de Barcelona

Josep Mª Espadaler i Gamissans

Universitat Autònoma de Barcelona

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Summary

Agraïments

El camí d'aquest treball ha sigut tan llarg i ple d'entrebancs, que em sembla quasi impossible haver arribar al final. Tot i així, al llarg de tots aquests anys (molts!) he tingut la sort de comptar sempre amb l'ajut i suport de molta gent. A tots vosaltres, i espero no deixar-me a ningú, us he d'agraïr tant el haver estat al meu costat i haver confiat en què algun dia acabaria!!! Fins i tot jo no m'ho acabo de creure i sense tots vosaltres mai no hagués estat capaç.

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INTRODUCTION

1. ELECTRODIAGNOSIS IN VETERINARY NEUROLOGY

Electrodiagnostic testing, which involves evaluation of the responses of skeletal muscles to electrical stimulation, was introduced into veterinary medicine in 1949[1]. However, because of limitations of electronic instrumentation for recording electric activity of muscles, electromyography did not develop as an independent field until 1967[2]. Since then, the application of electrophysiological diagnostic techniques has become a cornerstone in the investigation of neuromuscular diseases in veterinary neurology. The purpose of electrodiagnostic testing is to demonstrate the presence, absence, reduction or abnormality of the contractile response of a muscle to electric stimulation of its motor nerve, or to demonstrate quantitative or qualitative alterations of the electric activity of a muscle in the resting state. The instrument used to record the electric activity of muscles is the electromyograph. Electrical activity from striated muscles is recorded by two electrodes (anode and cathode), the signal obtained is then amplified and visually displayed in the electromyograph oscilloscope in the form of motor unit potentials or spontaneous electrical activity. In addition, motor or sensory nerves can be stimulated by a second pair of electrodes, the compound motor unit (motor nerve stimulation) or nerve potentials (sensory nerve stimulation) evoked in the corresponding muscle or nerve can then be recorded in the same way and displayed in the electromyograph oscilloscope.

The main electrodiagnostic techniques currently employed in veterinary neurology include:

1. **Electromyography**, the recording and study of the electrical activity of striated muscles, mainly used to assess skeletal muscle function.
2. **Electroneurography** or measurement of **motor and sensory nerve conduction velocities**, used to determine the speed at which an impulse is transmitted along a nerve, which evaluates the integrity and functionality of motor and sensory nerve fibers of the peripheral nervous system.
3. **F wave** studies, used in the assessment of motor conduction along the most proximal peripheral nerve segments, including nerve roots.
4. **Repetitive stimulation** of motor nerves, the most common test for investigation of neuromuscular transmission, primarily used to evaluate the functionality of neuromuscular junctions.
5. **Somatosensory and motor spinal cord evoked potentials**, which provide useful information about the functionality of both, the motor and somatosensory spinal cord pathways.
6. **Electroretinography and visual evoked potentials**, which evaluate the visual pathways, and
7. **Braintem auditory evoked potentials**, which assess the integrity and function of the auditory component of the eighth cranial nerve and the short latency central auditory pathways, located in the brainstem and midbrain.

The electromyographic study of spinal and brainstem reflexes in animals has been limited to experimental studies in dogs[3-7] and cats[8-12], but has received little clinical application in veterinary medicine. In addition, electrodiagnostic tests to assess other cranial nerves and/or brainstem function are currently inexistent in veterinary neurology. Many neurologic diseases can affect different cranial nerves and

brainstem areas in small animals as well as in horses, causing neurologic dysfunction difficult to evaluate, interpret or quantify clinically.

2. THE BLINK REFLEX: A NOCICEPTIVE REFLEX

Reflexes are stereotyped responses to stimuli. They require two or more neurons in series, a sensory (afferent) and a motor (efferent) neuron, and varying numbers of interneurons. The sensory neuron receives and transmits the stimulus to the motor neuron, located in the central nervous system. The stimulated motor neuron in turn, transmits impulses to the corresponding effector organ or muscle to induce the appropiate response to the initial stimulus. There are two main categories of spinal reflexes. The first category is of **postural reflexes**; these allow the animal to maintain its posture. The stimuli which initiate these reflexes are the streching of muscle, which elicits a stretch reflex and pressure of the skin of the foot, which elicits an extensor thrust. The second category is of **protective or nociceptive reflexes**. These are reflexes that protect the animal from injury. The types of stimulus which elicit these reflexes include mechanical irritation and pain.

i. Nociceptive reflexes

The three main nociceptive reflexes are the flexor withdrawal reflex, which causes withdrawal of a limb away from a painful stimulus, the abdominal guarding or superficial abdominal reflex, which protects the abdominal contents against mechanical disturbance, and the blink reflex, which protects the eyeball from external harmful stimuli. The afferent pathway of nociceptive reflexes is formed by

cutaneous sensory nerves belonging to mechanoreceptive, wide dynamic range or nociceptive neurons that can carry mechanical as well as nociceptive or painful information. The efferent limb of the reflex is formed by spinal or brainstem motor nuclei and their corresponding motor axons. The motor responses obtained when eliciting these type of reflexes are protective motor responses directed against potential harmful stimuli.

ii. History of the Blink Reflex

The Blink Reflex was first described in 1896 by **Walker Overend**, a British human physiologist, who described "a new cranial reflex" in a letter to the editor of *Lancet*[13]. In this first description, Overend reported that when the skin of the forehead was gently tapped with a stethoscope, the lower eyelid twitched on the same side, and that slight tapping in the middle line of the forehead was followed by twithing in both eyelids. In 1901, **Daniel Joseph McCarthy**, an American neurologist, re-described the reflex elicited by tapping the skin overlying the supraorbital nerve with a percussion hammer. McCarthy noted that a tap on either side of the face usually elicited a bilateral contraction of the orbicularis oculi muscles, and concluded that the supraorbital reflexes were "pure nerve reflexes", identical to tendon reflexes[14]. The exact mechamism of the blink reflex remained obscure until **Eric Kugelberg**´s electrophysiologic analysis in 1952. Using two differential amplifiers and a dual-trace oscilloscope, Kugelberg recorded the electrical potentials elicited in the orbicularis oculi muscles by tapping the skin in the outer corner of the eye with a metal rod. Kugelberg´s observations confirmed that the blink reflex is bilateral, that some part of the reflex pathway passes over the

spinal tract of the trigeminal nerve and that blink reflexes are abolished by general anesthesia and trigeminal rhizotomy[15]. In 1962 **Geoffrey Rushworth** confirmed that the receptors for the reflex were in the supraorbital nerve, but concluded that the blink reflex was a myotatic reflex, originating from proprioceptive receptors in the facial musculature[16]. Eight years later, **Bhagwan Shahani** demonstrated that the blink reflex is cutaneous in nature, and described the absence of muscle spindles in the facial muscles[17]. After these initial descriptions and observations, many different neurologists and neurophysiologists have re-described the technique for eliciting and recording the blink reflex, and have widely demonstrated its uselfulness in the diagnosis and definition of many neurologic diseases affecting both, the central and the peripheral nervous system.

2.1. THE ELECTRICALLY ELICITED BLINK REFLEX: ELECTROMYOGRAPHIC COMPONENTS AND ANATOMIC PATHWAYS

The blink reflex is a contraction of the eyelids in response to stimulation of the skin of the face. The afferent pathway of the reflex is formed by sensory fibers in the trigeminal nerve supplying the skin of the head. Motor fibers in the facial nerve innervating the orbicularis oculi muscles constitute the efferent pathway[15-18]. The response obtained after a unilateral stimulus of the trigeminal nerve is usually a bilateral contraction of the orbicularis oculi muscles. The blink reflex can be electromyographically recorded. In humans, reflex blinks are usually evoked by stimuli such as a mechanical tap or electrical current delivered to the skin in the periorbital region. The reflex responses of the orbicularis oculi muscles are then recorded by electrodes placed bilaterally in the eyelids so they can be further

evaluated. Electromyographic (EMG) recordings from the orbicularis oculi muscles show that the blink reflex evoked by electrical stimulation of the supraorbital (trigeminal) nerve comprises two responses, a first, unilateral response and a late, bilateral reflex response (Figure 1).

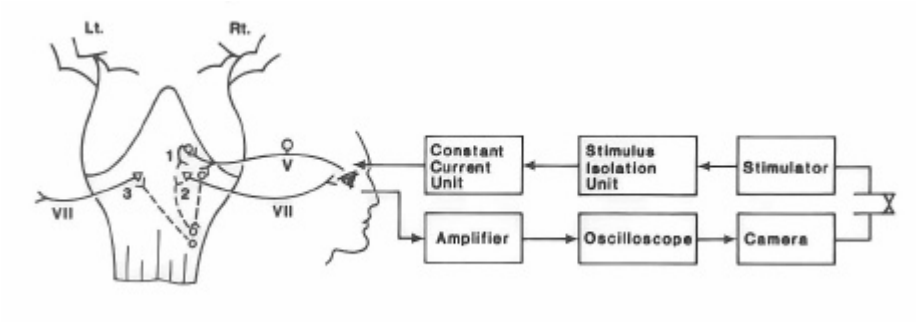

Figure 1. Stimulation and recording arrangement for the blink reflex in human beings, with the presumed pathway of R1 through the pons (1) and ipsilateral and contralateral R2 through the pons and lateral medulla (2 and 3). *Kimura, 2001 (Ref. 18)*

The first or early reflex, R_1, is a short EMG response (not visible clinically) that occurs at a 10-15 ms latency ipsilateral to the side of the stimulation. The second or late electromyographic response, R_2, has a 30-40 ms latency, is bilateral and more prolonged[18,19] (Figure 2).

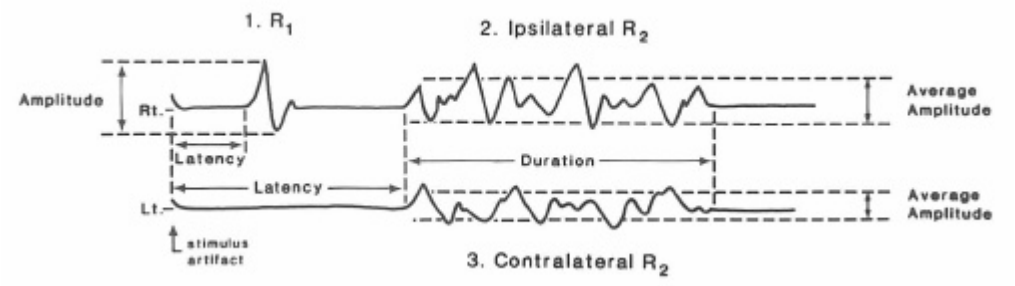

Figure 2. A typical oscilloscope rcording of the blink reflex after right-sided stimulation. Note an ipsilateral R1 response and bilateral, simultaneous R2 responses. *Kimura, 2001 (Ref.18)*

The two R_2 responses (ipsi- and contralateral) occur synchronously, with the latency of the contralateral one being slightly longer than that of the ipsilateral[20]. The R_2 response is clinically observed as the actual blink[17,21,22]. The afferent impulses for the R_1 blink reflex are conducted by medium-myelinated (A-β) trigeminal fibers and relayed through a short oligosynaptic circuit (from 1 to 3 interneurons) to the facial motoneurons. The whole circuit lies in the pons. The R_2 blink reflex is mediated by low-threshold A-β and possibly A-δ trigeminal afferents. Nerve impulses responsible for R_2 are conducted through the spinal tract of the trigeminal nerve in the dorsolateral region of the pons and medulla oblongata before they reach the most caudal area of the spinal trigeminal nucleus. From there, impulses are further relayed through polysynaptic medullary pathways ascending both ipsilaterally and contralaterally to the stimulated side of the face, before connecting to the facial nuclei. Impulses cross the midline in the caudal medullary region. The trigeminofacial connections ascend through the lateral tegmental field, lying medial to the spinal trigeminal nucleus[19,23] (Figure 3).

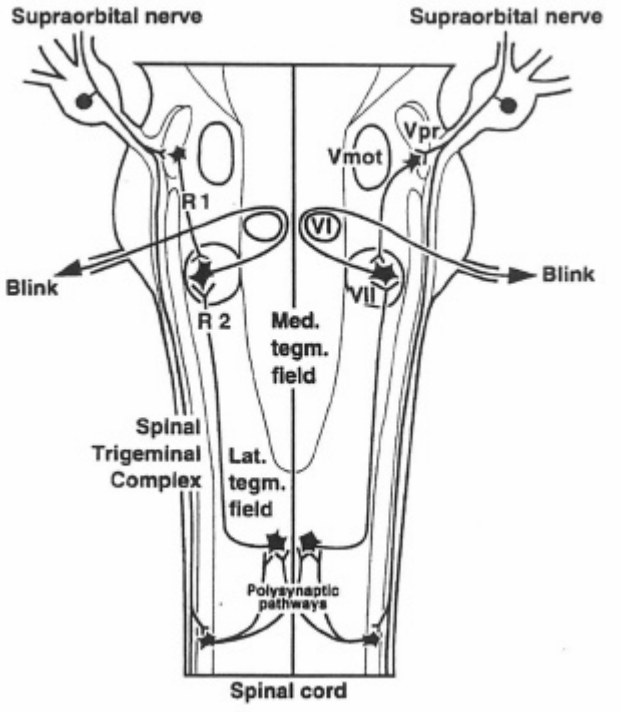

Figure 3. Schematic drawing of the blink reflex circuits. Afferents for the R1 blink reflex connect with an oligosynaptic chain of interneurons close to the ipsilateral trigeminal principal sensory nucleus (Vpr). The afferents for the R2 blink reflex descend along the spinal trigeminal tract and connect with a polysynaptic chain of interneurons located in the lateral tegmental field of lower medulla. V mot, trigeminal motor nucleus; VI, abducens nucleus; VII, facial nucleus.

Cruccu, 2000 (Ref 19)

In addition, R_2 seems to receive supratentorial influences[24-28]. R_1 and R_2 share the same facial motoneurons. A third late response (R_3) may appear after R_2 when high-intensity stimuli are applied to the supraorbital nerve. This reflex response appears to be relayed through small diameter, high-threshold trigeminal afferent fibers, perhaps nociceptive ones, and a longer pathway involving the caudal portion of the medulla oblongata and cranial end of the cervical spinal cord. It seems to be related to pain sensation and appears inconsistently[29-31].

2.2. CLINICAL APPLICATIONS OF THE ELECTROMYOGRAPHYCALLY RECORDED BLINK REFLEX

Of the two components of the human blink reflex, R_1 is a strictly segmental response, rather stable, resistant to all suprasegmental influences, including supratentorial lesions, disorders of consciousness and cognitive factors[24-28,32-38]. R_1 is diagnostically highly sensitive in extra-axial lesions. In human clinical neurology, therefore, R_1 is used most often to investigate the afferents from the supraorbital region, to assess conduction of the fifth and seventh cranial nerves and in the diagnosis of pontine lesions[18,19]. In contrast, the brainstem interneurons that mediate R_2 seem extremely sensitive to all sorts of sensory inputs (an R_2-like reflex can be elicited by sounds, flashes of light, radiant heat pulses, and electrical or mechanical stimuli delivered anywhere to the face or even to distant regions)[23,39-42]. Possibly because of the high number of synapses in the reflex circuit, R_2 is relatively unstable, habituates rapidly to repetitive rythmic stimulation, and is strongly modulated by suprasegmental influences, cortical and basal ganglia dysfunction, disorders of consciousness, and cognitive factors[24-28,32-37]. Although R_2 is less

reliable than R_1 in disclosing peripheral lesions, the simultaneous recording of the bilateral R_2 allows differentiation between damage to the afferent (trigeminal) and efferent (facial) pathways of the reflex[18,19]. Furthermore, R_2 alone is usually abnormal in lateral medullary lesions[43-46].

i. Peripheral trigeminal and facial nerve disorders

The electrically elicited blink reflex has been proven to be an excellent diagnostic tool in the evaluation of several trigeminal and facial neuropathies[18-20,47-52]. The blink reflex, which examines the whole course of the facial nerve, may provide valuable information regarding the extent and location of the lesion in most facial neuropathies (eg, Bell´s palsy)[53-60]. Most patients with facial nerve lesions show abnormalities of the R_1 and R_2 components in the ipsilateral orbicularis oculi muscle, regardless of the side of stimulation (**efferent abnormality**)[18,19,55] (Figure 4).

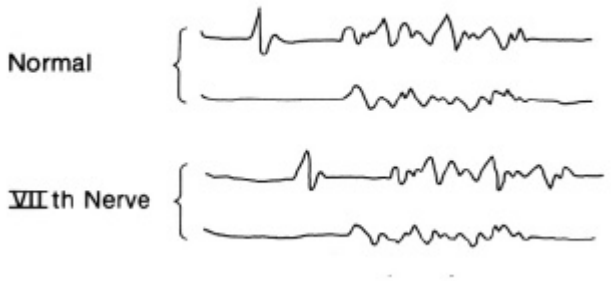

Figure 4. Blink reflex abnormality. The finding suggests the conduction abnormality of the efferent pathway along the facial nerve. *Kimura, 2001 (Ref. 18)*

In addition, serial evaluation of the delayed or absent reflex responses may provide highly valuable information regarding the severity and prognosis of the disease[18,55]. The blink reflex may also be used to test conduction of the trigeminal nerve, which constitutes the afferent arc of the reflex pathways. In these cases, when the affected side of the face is stimulated, a bilateral delay or absence of blink reflex responses can be observed (**afferent abnormality**)[18-20,55] (Figure 5).

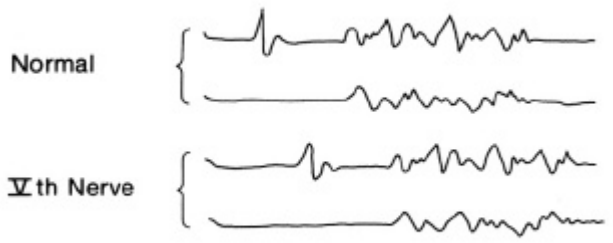

Figure 5. Blink reflex abnormality. The finding suggests the conduction abnormality of the afferent pathway along the trigeminal nerve. *Kimura, 2001 (Ref.18)*

ii. Brainstem dysfunction

Alterations of the blink reflex responses might as well be due to lesions in the brainstem affecting the central connections of the reflex pathways. The abnormalities found in human patients are variable depending on the specific location of the lesion within the brainstem[24,61-68]. In general terms, a delay of R_1 appears to be relatively specific to pontine involvement because of intrinsic (eg, multiple sclerosis) or extrinsic (eg, cerebellopontine angle tumors) lesions[69-73].

Although R_2 might be altered by pontine lesions, it seems to be more consistently affected by lateral medullary lesions (eg, Wallenberg syndrome)[43-46].

iii. Hemispheric lesions

The exact central pathway of the blink reflex remains unknown. Several studies suggest that the blink reflex utilizes trigeminofacial connections and that suprasegmental structures act directly on the facial motor nucleus to modify the components of the blink reflex[28]. Thus alteration of R_1 does not necessarily indicate a pathologic process of the reflex arc itself, because edema or other lesions outside the brainstem can also cause conduction abnormalities. A reversible block of R_1 seen in comatose patients ususally results from acute supratentorial lesions or from massive drug intoxication[35]. The results of some studies show that suprasegmental structures act directly on the facial motor nucleus to modify the components of the blink reflex[35]. Other authors propose that the R_2 response of the blink reflex is generated through longer pathways going up to the opposite thalamus or cortex, where they project down to bilateral facial nuclei[27]. Interruption of ascending pathways passing through the thalamus in hemispheric lesions as well as interruption of descending cortical facilitatory pathways would be the two potential explanations for the blink reflex abnormalities found in patients with hemispheric lesions[28].

iv. Evaluation of extra-pyramidal disorders (man)

Habituation is a wide-spread phenomenon that has been defined as a gradual quantitative diminution of response to repeated uniform stimuli[74]. Habituation of the blink reflex can be observed electromyographically as diminution of size of its second component, R_2 (the polysynaptic nociceptive component of the reflex), but not of R_1. When stimuli are delivered repeatedly at a constant frequency, the R_2 response gradually decreases in size and eventually disappears[18,19,74,75]. R_2 readily habituates in normal human beings, but not in patients with Parkinson´s disease[16,75]. Similarly, the blink reflex fails to show physiologic habituation in nocturnal myoclonus. On the other hand, R_2 shows enhanced habituation and an increased latency in patients with Huntington´s disease[76,77]. These findings are believed to be caused by a lack of inhibition (parkinson´s disease)[75] or lack of facilitation (Huntington´s disease)[76,77] from suprasegmental structures on the brainstem interneuronal pool controling the R_2 blink reflex responses[18,19].

3. DIRECT FACIAL MOTOR NERVE STIMULATION

The electrically elicited blink reflex tests both, the trigeminal (afferent) and facial (efferent) nerves. Testing the reflex arch, latencies of the blink reflex components reflect the conduction along the entire facial nerve including both, the intraosseus and the peripheral portion[18]. Performing direct electrical stimulation of the facial nerve distally (after its exit from the petrous portion of the temporal bone) and electromyographically recording the direct motor-evoked potentials obtained in the orbicularis oculi muscles, distal facial nerve conduction can be assessed[18]. The

electromyographic potential recorded in the orbicularis oculi muscle upon direct facial nerve stimulation is a typical bi- or triphasic compound muscle action potential with a highly stable latency. Reported normal values for facial nerve latencies in adult human beings range from 3 to 5 ms[18]. Amplitude values vary greatly with stimulus intensity. Therefore, comparisons between amplitudes obtained on both sides of the face are commonly used instead of absolute amplitude values[18,53-57]. In some cases, a second electromyographic potential can be observed after the direct response upon direct facial nerve stimulation. This second electromyographic deflection is a reflex response thought to be caused by stimulation of sensory fibers in the facial nerve that terminate in spinal trigeminal nucleus neurons which, in turn, activate facial nucleus motoneurons innervating the orbicularis oculi muscles[78-81]. In human beings, latency and amplitude of the direct facial muscle-evoked potential are commonly used in the assessment of peripheral facial nerve disorders (eg, Bell´s palsy)[53-57]. By evaluating both, the latency of the R_1 component of the blink reflex and the latency of the direct facial response, a comparison can be made between the conduction through the distal facial nerve and that through the entire reflex arch, determining if facial nerve dysfunction occurs proximally (intraosseous portion) or more distally.

4. PREVIOUS STUDIES IN DOMESTIC ANIMALS

The blink mechanisms and the anatomical structures and pathways involved in the reflex have been extensively evaluated in cats[78-80,82,83], which have commonly been used as animal research models in human neurophysiology. Several facial reflexes were also previously evaluated and described in dogs[84]. In this study,

electromyographic recordings were obtained in the orbicularis oculi and other facial muscles after electrical stimulation of different branches of the trigeminal and facial nerves. Direct facial nerve motor evoked potentials and reflex trigemino-facial potentials were recorded and characterized. However, this study (as well as all the previously reported studies in cats) were performed in anesthetized animals. It is well known that anesthesia and level of consciousness affect and can abolish the R_2 component of the electromyographically recorded blink reflex through suppression of the polysynaptic brainstem pathways involved in the genesis of this late component[17,32-36]. Therefore, late blink reflex components (ipsi- and contralateral) could not be fully evaluated. As mentioned before, evaluation of the bilateral late components of the blink reflex is essential to differentiate between lesions of the afferent (trigeminal) and efferent (facial) pathways of the reflex, as well as to assess caudal brainstem function and the influences of supratentorial structures on lower brainstem areas.

5. AIM OF THIS STUDY

There are many diseases that can affect the brainstem focally or in a diffuse manner in domestic animals (brain neoplasms, infectious and non-infectious meningoencefalitides, degenerative diseases). In addition, several neuropathies have been described affecting the facial and/or trigeminal nerves in dogs and horses (idiopathic facial palsy and trigeminal neuritis in dogs, traumatic facial paralysis in horses, infectious and non-infectious neuritides, peripheral nerve neoplasms and metabolic neuropathies in both species). Modern imaging techniques such as magnetic resonance imaging (MRI) allow structural and anatomical evaluation of

both, brainstem and cranial peripheral nerves, but they do not provide any information about functionality of the imaged areas. Electrophysiological techniques provide functional evaluation of the nervous system, even in the absence of anatomical or structural lesions. Clinical electrophysiologic tests currently employed in veterinary medicine are highly useful in the functional assessment of peripheral spinal nerves, spinal cord and the auditory brainstem pathways, however no specific electrodiagnostic test is currently available to evaluate the facial and/or trigeminal nerves as well as the mesencephalic, pontine and medullary brainstem areas. For these reasons, electrophysiologic assessment of the blink reflex in non-anesthetized animals could be a highly valuable diagnostic tool in veterinary neurology.

In addition, the electrically elicited blink reflex test is commonly used in human neurology in the evaluation of disorders of voluntary movement of extra-pyramidal origin, such as Parkinson´s disease or Huntington´s chorea. The study of the reflex in domestic animals could also be a valuable experimental tool in human neurophysiology and could serve as a basis for future experimental studies.

OBJECTIVES AND STUDY PROPOSAL

1. To test electromyographycally the blink reflex in dogs and horses, evaluate the responses obtained and compare them with those observed in human beings. To report normal latency values and electromyographic characteristics (amplitude, duration, habituation) for the different components of the electromyographically recorded blink reflex in dogs and horses.

2. To obtain electromyographic recordings in the orbicularis oculi muscles elicited by direct facial motor nerve stimulation in dogs and horses and to report the characteristic features of the motor-evoked potentials recorded in these muscles.

3. To evaluate the uselfulness of the electrically elicited blink reflex and direct facial motor nerve stimulation in the diagnosis and evaluation of peripheral facial and trigeminal nerve disorders in dogs and horses.

PUBLISHED ARTICLES

Article 1

Electrically induced blink reflex in horses

Article 2

Electrically induced blink reflex in horses with trigeminal and facial nerve blocks

Article 3

Electrically induced blink reflex and facial motor nerve stimulation in Beagles

DISCUSSION

1. BLINK REFLEX TEST

As in humans, the blink reflex elicited by electrical stimulation of the trigeminal nerve in dogs and horses consisted of two separate electromyographic reflex responses, which could be consistently recorded in the orbicularis oculi muscles. The first reflex response (R_1) was a polyphasic muscle potential of highly stable latency in both species. It was highly reproducible and constant and it appeared only in the orbicularis oculi muscle ipsilateral to the stimulated side. Bilateral late reflex responses (ipsilateral R_2 and contralateral R_c) were also consistently elicited in both species. These second reflex responses were polyphasic muscle potentials of longer and more variable latencies than that of R_1. In dogs, but not in horses, these second reflex potentials showed longer durations and smaller amplitudes than R_1. A third ipsilateral response (R_3) was inconsistently observed in 85-90% of the horses and 60% of the dogs studied. R_3 was also a polyphasic muscle potential of highly variable, and always longer latency than that of R_2. No significant differences between sides of stimulation were observed for the latencies of any of the blink reflex responses.

The R_1 response of both, dogs and horses, had a lower stimulation threshold and a more stable latency than R_2 (Table 1). The R_1 latency represents the conduction time along the trigeminal and facial nerves and pontine synaptic relay[18]. However, neuromuscular delay and conduction along the orbicularis oculi muscle fibers might also have a small contribution to this latency time.

Latency	**Horses** **n = 10**	**Dogs** **n = 15**
Left	12.9 ±1.11	7.36±0.86
Right	12.35±1.12	7.22±0.54

Table 1. Mean latencies (±SD), in milliseconds, of the R_1 blink reflex responses in dogs and horses.

As in humans, the second responses of dogs and horses had a more variable latency than R_1, and the contralateral late response (R_c) showed always a slightly longer latency than the corresponding ipsilateral R_2 (Table 2). This is to be expected for a reflex response that is known to be transmitted through polysynaptic reflex pathways involving the trigeminal spinal and sensory nuclei, the contralateral thalamic nuclei or cortex, and the bilateral facial nuclei[44].

	Latency	**Horses** **n = 10**	**Dogs** **n = 15**
R_2	**Left**	44.05±3.77	33.65±3.47
	Right	43.74±6.19	33.84±2.86
R_c	**Left**	51.53±5.92	42.5±7.72
	Right	49.57±6.06	43.44±6.57

Table 2. Mean latencies (±SD), in milliseconds, of the R_2 and R_c blink reflex responses in dogs and horses.

Of the two components, R_1 would seem better suited to assess conduction along the trigeminal and facial nerves. R_2 would be less reliable for measuring conduction time because its inherent variability in latency from one trial to the next. This high variability appears to be related to the excitability of interneurons and delay of synaptic

transmission in addition to the axonal conduction time[18]. However, analysis of the R_2 response would be essential in determining whether the afferent or efferent part of the reflex pathway, or the brainstem is primarily affected[43,55]. As observed in humans[18,19,74,75], both late responses (R_2 and R_c) habituated. They gradually decreased in amplitude and eventually disappeared with repeated stimuli when those were given too frequently or were of too high intensity. In order to avoid habituation, the interstimulus interval was kept over 30 seconds in all studies. The late responses (R_2 and R_c) are highly influenced by level of consciousness or state of arousal in man, and they disappear under general anesthesia or even under some types of sedation (eg, diazepam), because of central blocking of the multysynaptic reflex pathway[24,32-38]. If any of the animals, but more consistently the dogs, would fall asleep during the experimental procedure, the contralateral late response (R_c)was the first one to fade, followed by the ipsilateral one (R_2). The first response (R_1) never disappeared. After arousal, both late responses reappeared.

The third response (R_3) appeared inconsistently in 9 of the 15 dogs and more consistently in 27 of the 31 horses tested. This third late potential is induced in humans by activation of high small-diameter, slow-conducting, high threshold afferent fibers, which are less excitable than those responsible for R_2[29]. R_3 has, in humans, a longer latency than R_2 and appears only after stimulations of high intensity[29,30]. There appears to be a significant correlation between pain sensation and R_3 threshold. The fact that in horses R_3 appeared more regularly after R_2, could mean that in this species the afferent fibers responsible for R_2 and R_3 might belong to the same group or have more similar thresholds than they do in humans or dogs. This phenomenon migh also be interpreted as a reinforcement of the defensive nature of the reflex in prey animals. In both, dogs

and horses, R_3 showed a highly variable and long latency (Table 3), indicating that it is also possibly transmitted through slow-conducting, unmyelinated fibers and longer, multysynaptic central pathways.

Latency	**Horses** **n = 10**	**Dogs** **n = 15**
Left	82.41±8.56	59.41±26.4
Right	82.28±10.25	58.62±22.84

Table 3. Mean latencies (±SD), in milliseconds, of the R3 blink reflex responses in dogs and horses.

The mean latencies of all the recorded muscle potentials are shorter in dogs and longer in horses than the corresponding latencies in humans, probably because of the shorter (dogs) and longer (horses) length of the trigeminal and facial nerves in these species than in humans. Latencies of onset of the evoked muscle potentials would be expected to be shorter in smaller dog breeds and longer in larger dogs.

2. DIRECT FACIAL NERVE STIMULATION

Electrical stimulation of the facial (dogs) or auriculopalpebral (horses) nerve elicited a direct electromyographic response (D) in the ipsilateral orbicularis oculi muscle of all animals tested. This direct response was a highly consistent, byphasic or polyphasic compound motor unit potential. A second facio-facial reflex response (RF) appeared after the direct response in the ipsilateral orbicularis oculi muscle of all dogs and 7 of the 10 horses tested. No significant differences between sides of stimulation were detected for the latencies of the direct and reflex facial responses in any species.

The direct compound muscle evoked potential elicited by stimulation of the facial nerve showed a very stable latency, always shorter than that of R_1 (Table 4).

	Latency	**Horses** **n = 10**	**Dogs** **n = 15**
D	**Left**	3.37±0.73	2.36±0.35
	Right	3.24±0.62	2.42±0.43
RF	**Left**	16.72±1.98	15.1±1.66
	Right	16.65±2.12	14.4±1.84

Table 4. Mean latencies (±SD), in milliseconds, of the direct (D) and reflex (RF) facial responses in dogs and horses.

Evaluation of the latency of the direct response obtained upon stimulation of the facial nerve provides a measure of distal facial nerve conduction, more precisely, a measure of conduction of the fastest fibers within the distal facial nerve. By evaluating both, the latency of the R_1 blink reflex response and the D latency, comparisons can be made between the conduction through the distal facial nerve and that through the entire reflex arch (which includes the trigeminal nerve and the proximal segment of the facial nerve), determining if facial nerve dysfunction occurs proximally (intraosseous portion) or more distally[18,47,54,55]. With a lesion of the proximal (intraosseous) portion of the facial nerve, one would expect an abnormally long R_1 latency and normal or mildly delayed D latencies, providing a high R_1/D ratio. Whereas a low R_1/D ratio would be consistent with slowing of distal facial nerve conduction. Another parameter regularly measured in compound muscle evoked potentials is their amplitude. Amplitude of the D response is an indicator of the number of axons conducting impulses in the facial nerve and it

increases with increasing stimulus intensity up to a maximum[18,53]. Amplitudes of the D responses showed extensive variation among all the animals tested (as they do in humans). Thus, as in man[56], comparisons between amplitudes on both sides of the face should provide a more sensitive measure of nerve function, rather than absolute amplitude values. In man, a D amplitude ratio of the side affected in idiopathic facial paralysis to the healthy side of less than 50% is suggestive of distal facial nerve degeneration, and a ratio of less than 25% indicates a poor prognosis for recovery of function[57].

The reflex facio-facial response that appeared after D upon direct facial nerve stimulation, showed a longer and slightly more variable latency than that of the direct response (Table 4). The reflex facio-facial response observed in dogs and horses has also been described in humans[81]. The afferent fibers responsible for this reflex muscle potential could be antidromically stimulated facial motor fibers, trigeminal branches activated through trigeminofacial anastomoses, or sensory fibers running in the facial nerve. Results of studies in man[79,81] and cats[78,80] indicate that the afferent fibers of the facio-facial reflex run in the facial nerve. The central termination of these sensory fibers is thought to be in the spinal trigeminal nucleus[79]. Then, the trigeminal motor neurons activated by these facial afferents would, in turn, activate facial nucleus motor neurons innervating the orbicularis oculi muscles and generate the reflex facio-facial response.

3. BLINK REFLEX TEST AFTER LEFT SUPRAORBITAL NERVE BLOCK

In both, dogs and horses, no reflex responses could be elicited on either side of the face upon stimulation of the blocked nerve at a normal stimulation level. Typical reflex

responses were elicited ipsilaterally and contralaterally upon stimulation of the right supraorbital nerve in all animals tested. The lack of responses upon left supraorbital nerve stimulation after the nerve block is consistent with the results in humans with lesions of the afferent (trigeminal) pathway of the reflex[20,24,48,55,85]. Lack of afferent impulses (complet axonal loss) should cause lack of responses upon stimulation of the affected side of the face, whereas delay of the responses or decreased amplitudes should be seen in partial axonal or demyelinating lesions[18,20,48,55,70,85].These results demonstrate that the electrically elicited blink reflex test can be useful in dogs and horses in the assessment of peripheral trigeminal nerve function.

4. BLINK REFLEX TEST AFTER RIGHT FACIAL (DOGS) OR AURICULOPALPEBRAL (HORSES) NERVE BLOCK

After the right facial or auriculopalpebral nerves were blocked, no blink reflex responses could be elicited in the right orbicularis oculi muscle upon stimulation of either left or right supraorbital nerves at any stimulation intensity. Typical responses were recorded in the contralateral side after stimulation of both, right and left supraorbital nerves. These results are consistent with those observed in humans with complete unilateral peripheral facial lesions and demonstrated that the blink reflex test can be useful in dogs and horses to assess peripheral facial nerve function. When the efferent limb of the reflex pathway is abolished, no responses can be evoked on the affected side, regardless the side of stimulation. With incomplete, partial axonal or demyelinating lesions of the facial nerve, delay of the responses and decreases in amplitude of the blink reflex potentials are observed on the affected side after ipsilateral

and contralateral stimulation[47,48,53-56,70,85]. In these instances, direct conduction latency of the facial nerve and amplitude of the direct potential may be of prognostic value.

5. PERSPECTIVE USEFULNESS OF THE BLINK REFLEX TEST AND DIRECT FACIAL NERVE STIMULATION IN VETERINARY NEUROLOGY

The results of our study indicate that both, the blink reflex test and direct stimulation of the facial nerve should be useful in determining prognosis for recovery of function in cases of facial nerve dysfunction by determining the level and extent of the facial nerve lesion, in differentiating central (brainstem) from peripheral involvement in cases of trigeminal and facial nerve dysfunction, in demonstrating the presence and extension of brainstem lesions in animals with multiple cranial nerve dysfunction due to degenerative, inflammatory or neoplastic brainstem lesions, in the evaluation of the effects of hemispheric lesions on brainstem function, and in the assessment and monitoring of brainstem and cerebral function in comatose patients.

CONCLUSIONS

1. The blink reflex can be induced in sedated dogs and horses by electrical stimulation of the supraorbital (trigeminal) nerve. Simultaneous bilateral, electromyographic recordings of the blink responses in the orbicularis oculi muscles allow evaluation of the different components of the reflex in these species.

2. Direct electrical stimulation of the facial nerve can be performed at the zygomatic arch (auriculopalpebral nerve) in horses and at the level of the stylomastoid foramen in dogs. The compound motor unit potentials evoked in this way can be electromyographically recorded in the orbicularis oculi muscles ipsilateral to the side of stimulation.

3. The electrically induced blink reflex in horses has two components. A first, ipsilateral R1 component, and a late, bilateral R2 component. A third component, R3, appears very frequently after R2, ipsilateral to the side of stimulation.

4. In horses, all blink reflex responses are polyphasic muscle potentials of variable amplitude and duration. R1 shows a highly stable latency. The late R2 responses have longer and more variable latencies than R1, and that of the contralateral response is always slightly longer than that of the ipsilateral one. The third R3 response shows the longer and more unstable latency of the three reflex responses.

5. Direct stimulation of the auriculopalpebral nerve in horses induces a biphasic-polyphasic muscle potential in the ipsilateral orbicularis oculi muscle, frequently followed by a reflex biphasic-polyphasic, facio-facial potential. The amplitude of

the direct facial evoked muscle potentials is highly variable. The first muscle potential shows a highly stable latency, always shorter than that of R1. The second facio-facial response has a fairly stable, but always longer latency that that of the direct response.

6. The electrically induced blink reflex in dogs has two components. An early, ipsilateral R1, and a late, bilateral R2. A third R3 reflex response can be observed in some dogs ipsilateral to the stimulated side, but this is not a common finding.

7. In dogs, all blink reflex responses are polyphasic muscle potentials. The R2 responses consistently show smaller amplitudes and longer durations that those of R1. As in horses, R1 is very stable in latency. The late responses have always longer and more variable latencies than that of the first response. The third response is inconsistent and shows a very long and unstable latency.

8. In dogs, electrical stimulation of the facial nerve elicits a direct response in the ipsilateral orbicularis oculi muscle, always followed by a second, reflex facio-facial response. The first is a biphasic-polyphasic potential and the second is a polyphasic muscle potential. Both facial responses show a highly variable amplitude. The first response has a very stable and short latency (always shorter than that of R1), whereas the second response has always a longer and slightly more variable latency than the first one.

9. As in humans, the late blink reflex responses (R2) of dogs and horses show habituation, a gradual quantitative decrease in amplitude with repeated uniform stimuli.

10. After experimental peripheral trigeminal lesions in both, dogs and horses, no blink reflex responses are observed on either side of the face upon stimulation of the lesioned nerve, but normal reflex responses can be elicited ipsi- and contralaterally upon stimulation of the normal (contralateral) trigeminal nerve.

11. After experimental peripheral facial nerve lesions in dogs and horses, no responses can be elicited in the ipsilateral orbicularis oculi muscle upon stimulation of either left or right trigeminal nerves. Normal reflex responses are observed in the contralateral orbicularis oculi muscle after stimulation of both right and left supraorbital (trigeminal) nerves.

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